EFFECT OF ROTATION, ORGANIC INPUTS AND TILLAGE ON CROP PERFORMANCE AND SOIL QUALITY IN CONVENTIONAL AND LOW-INPUT ROTATIONS IN CENTRAL IOWA

BY

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THESIS

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ABSTRACT

Even though the benefits of low external input (LEI) cropping systems to crops and soils are well recognized the specific links between cropping practices and associated soil quality and crop responses are not yet clear. In the Marsden plots in central Iowa, crop yields and input use efficiency have been increased by the use of longer and more diversified rotations and reduced chemical inputs. In this work we sampled roots and soil parameters at multiple dates and two depths in all cropping phases, in order to quantify changes in physical, chemical and biological soil quality indicators and root responses associated with tillage and cropping factors in a conventional and two LEI rotations of different lengths and including different legume species. Improvements in soil quality indicators and plant productivity were expected to be driven by the amount and placement of organic residues and to fluctuate with tillage and cropping phase. On a system basis, particulate organic carbon (POM-C) and potentially mineralizable nitrogen (PMN) were increased in both LEI rotations relative to a two year (2-yr) corn (*Zea mays* L.) -soybean (*Glycine max* L.) rotation. Biologically labile organic matter fractions were highly stratified in the 2-yr rotation compared to the LEI rotations and the lower depth of the 2-yr rotation was consistently depleted. Corn roots followed a similar pattern, being concentrated in the top depth in the 2-yr rotation while more fully exploring the profile in the LEI rotations. Low C:N ratios in the soybean roots in the LEI rotations suggest greater N availability in the LEI soybean phase. Soil parameters did not differ between LEI rotations even though the 3-yrrotation included red clover (*Trifolium pratense* L.) instead of alfalfa (*Medicago sativa* L.), a shorter rotation length, and significantly greater mean annual organic inputs than did the 4-yr rotation. Corn yield in the 3-yr LEI rotation was significantly higher than that achieved in the 2-yr conventional rotation, and soybean yield in the 4-yr rotation was higher than that in the 2-yr rotation. Seasonal sampling showed that 1) soil parameters fluctuated during the growing season but did not increase in response to particular cropping phases and 2) that the stratification observed in the 2-yr rotation was consistent over time for both corn and soybean. The

practice most responsible for increasing soil quality and plant performance in the LEI rotations appeared to be the deep incorporation of compost and green manures prior to corn production. This practice benefited both corn and soybean, primarily by increasing the amount and distribution of nutrients available to roots as evidenced by greater POM-C and PMN levels in the subsoil.

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Introduction

Low external input (LEI) cropping systems aim to reduce reliance on chemical pesticides and fertilizers by using longer, more diverse rotations, green manures and composts, and focused applications of chemical inputs to supply nutrients and control insect pests, weeds and diseases (Ball et al., 2005; Drinkwater and Snapp, 2007). Such systems aim to improve yield and system efficiency by coupling plant needs with soil processes to support those needs instead of supplying nutrients with chemical fertilizers and reducing competition with pesticides as is done in conventional systems (Liebman et al., 2008). When weeds are well controlled, cereal crops grown in LEI or organic systems can produce yields comparable to or higher than those achieved in simpler annual crop systems receiving greater chemical inputs (Liebhardt et al., 1980; Delate and Cambardella, 2004; Teasdale et al., 2007; Posner et al., 2008; Coulter et al., 2011).

While this “rotation effect” associated with length and crop diversity is partly attributable to pest and disease suppression (Smith et al., 2008), many argue these benefits are derived from improvements in soil quality, because increases in the quantity and or quality of soil organic matter are frequently observed in such systems (Wander et al., 1994; Ball et al., 2005, Karlen et al., 2006; Drinkwater and Snapp, 2007), and improved crop yields have been shown to be correlated to increases in soil quality indicators (Spargo et al., 2011). Longer rotations (Delate and Cambardella, 2004; Coulter et al., 2011), manure (Sainju et al., 2008), and cover crops (Wander et al., 1994) are known to be beneficial, but the optimal length of rotation, rate and frequency of manure addition, or species and frequency of cover crops are not known. Most studies of diversified farming systems tend to focus on yield outcomes or soil quality as endpoints, and do not attempt to evaluate the processes by which management practices change soil quality to relate those changes to plant performance or how those changes in plant response cycle back to alter the soil system.

As a generalization, the use of diversified rotations and addition of animal and green manures can alter system efficiency by changing the quantity, degree of incorporation and type of material entering the soil, and with this, presumably by altering the decay environment (Govaerts et al., 2009). How LEI system organic matter (OM) quantity, incorporation and type differ from conventional rotations depends on the system.

Use of manures and composts can increase the quantity of OM added in LEI rotations. Greater OM addition is often associated with increased soil OM (SOM) (i.e. Buyanovsky and Wagner, 1998; Aoyama et al., 1999; Ma et al., 2011). The presence of SOM is known to promote good soil physical, chemical and biological properties, increasing nutrient retention and system efficiency (Drinkwater and Snapp, 2007), and studies which observe soil quality under different rates of residue addition have found higher rates of residue return to be associated with higher SOM, lower bulk density, higher aggregate stability and higher microbial biomass (i.e. Karlen et al., 1994, Franzluebbers and Brock, 2006). However, SOM does not always increase with greater OM additions, particularly in very high organic-matter soils (i.e. Gulde et al., 2008, Chung et al., 2010) or under moldboard plow tillage (Moebius-Clune et al., 2008).

In addition to changing the quantity of inputs LEI rotations alter the pattern of input distribution and with this the intensity of soil disturbance. Studies that emphasize the role of crop and manure -based inputs overlook the fact that, in the Midwestern United States, to the extent that the LEI rotation aims to reduce external chemicals, these practices often require full-inversion tillage to completely kill the green manure crop, reduce weed competition, and incorporate manures to promote mineralization (Peigné et al., 2007). The negative effects of tillage on soils and soil organic matter are well known (i.e. Franzluebbers, 2010). How tillage-based disturbance interacts with other practices, including manure additions and different crops, is less well understood. Moldboard plowing, a dominant form of inversion tillage, interacts with the other management practices by affecting organic input mineralization rate and placement. It causes faster mineralization of SOM by breaking up protective structures (Six et al., 2002; Chung et al., 2008), increasing aeration (Topp et al., 2000) and putting residues in closer contact with the soil (Franzluebbers et al., 1998). The faster mineralization rates typically associated with tillage provide more N to the growing crop but can also degrade soil quality, particularly in the surface soil (Peigné et al., 2007; Franzluebbers, 2010). The effect of tillage varies by the degree to which the OM is protected and the activity of the microbial community; soils with very strong structure (Yoo and Wander, 2008), in a cooler climate (Angers et al., 1997) or whose prior OM concentration exceeds the protective capacity of the soil (Vandenbygaart, 2003) may not lose SOC when subjected to an increase in tillage. Tillage also, alters structure and moisture interactions in ways that influence decomposition; for example, organic matter may decay faster in surface of less-tilled soils where water is limiting, since less-tilled soils tend to hold more moisture (Franzluebbers and Arshad, 1996).

There may be other important depth-based effects of tillage that are overlooked. Since moldboard plow tillage tends to place residues towards the bottom of the plow depth (Allmaras et al., 1996)) soil quality and C storage can, in cool climates and heavy soils, be improved overall by C accumulation at or below the depth of plowing (Angers and Eriksen-Hamel, 2008). Tillage also has direct positive structural effects, temporarily loosening compacted or heavy soils. If soil strength is limiting, tillage in this case may promote OM accumulation by breaking up physical impedance to plant root growth, leading to root proliferation. It is important to deepen our understanding of the conditions under which tillage degrades or improves soil quality. A useful tool for looking at the effects of tillage on the soil environment is the stratification ratio; that is, the value of a soil quality in the surface soil divided by its value in the subsurface (Franzluebbers, 2002). Because tillage alters OM distribution and decay environments, an expression of the depth distribution may be more useful than a value for the total soil profile (Franzluebbers, 2002).

An important factor which interacts with the quantity and placement of organic material is the type of material added. LEI rotations frequently include species with extensive root systems, which contribute to soil quality through their influence on the decay environment and their intrinsic characteristics. Living roots contribute to improvements in soil structure and OM protection by drying the soil (Hallett et al., 2009), creating a richer microbial environment, particularly for fungi (Haynes and Beare 1997) and providing durable physical support around which aggregates can form and stabilize (Oades and Waters, 1991; Puget and Drinkwater, 2001). Roots also have been shown to be more recalcitrant in soil than shoots (Rasse et al., 2005) and have been shown to contribute a disproportionate amount of soil organic C (Balesdent and Balabane, 1996; Kong and Six, 2010). These findings suggest that extensively rooted crops such as alfalfa, small grains and clover have an important soil-building function in the rotation, which may counteract the destructive effect of tillage (Spargo et al., 2011). In addition, incorporation of composted manure, which is highly recalcitrant (Abiven et al., 2007), has been shown to increase the number and stability of soil pores (Ball et al., 2005), may have a mild but long-lasting aggregative effect (Abiven et al., 2009) and contribute greatly to the soil labile fraction (Poudel et al., 2002; Gulde et al., 2008). The LEI system practices of adding composts and more roots to the soil, whether by promoting soil structure and aggregate protection or by their own longer mean residence time, are expected to have observable effects on soil structure and OM content, and overall nutrient cycling efficiency (Puget and Drinkwater, 2001; Ball et al., 2005; Nyiraneza et al., 2010).

The goal of this study was to determine which if any of the mechanisms by which LEI management is predicted to improve soil quality are important in the finely-textured, high OM soils of Marsden Farm Cropping Systems Experiment. The trial, located near Ames, Iowa was started in 2002 and consists of a conventionally managed 2-year rotation (corn-soybean), as well as 3-year (corn-soybean-oat + red clover) and 4-year (corn-soybean-oat + alfalfa-alfalfa) rotations. Composted beef manure is incorporated into the 3-yr and 4-yr rotations in fall before corn. This site provides an opportunity to explore plant-soil relations in LEI systems that produce corn and soybean yields which equal or exceed those of their conventional counterparts (Liebman et al., 2008). Previous work suggests improved soil-based differences may be responsible but does not yet indicate changes in total SOC or N (Lammerding and Wander 2005, M. Liebman, personal communication, data not shown). The objectives of this study were to: 1) quantify the effects of cropping system and component practices (rotation length, residue and manure additions, and tillage) on soil quality and productivity and 2) evaluate the influences of specific crops and management events soil response and the health of the following crop. By sampling all crops in all rotations at two depths in the spring of 2009 and 2010, we aimed to describe long term differences which had developed between systems as a result of the different management packages. In addition, we sampled these rotations on multiple dates in the cropping season, to describe to what extent each crop growth environment was a legacy of the preceding crop's growth and decay characteristics and to capture the impact and duration of specific management events (Figure 1).

Improvements in soil quality indicators and plant productivity were expected to be driven less by the amount than the degree of incorporation and type of organic residues. Despite their more extensive tillage, in these heavy-textured soils LEI plots were expected to preserve greater OM than the 2-yr plots. Attendant structural benefits were expected to include a greater overall %WSA and decreased bulk density. Decreased bulk density was expected to be seen particularly under the recently-tilled corn. This difference was expected to be correlated with increased root length. Organic matter and aggregate stability were expected to be significantly increased under small grains and forage legume crops , offsetting the negative effects of tillage.

Methods

Timing and extent of sampling

To quantify inputs in order to test both system and temporal hypotheses we sampled above- and below-ground biomass in fall 2009 and generated rough C budgets and estimates of external N entering each system. Carbon inputs to a crop were calculated as the sum of measured C from the biomass and from the fall root mass from the crop preceding it in the rotation. This method assumes the 2009 values are representative. Data based on samples taken on a single date were used to evaluate plant productivity; corn and soybean biomass and grain from fall of 2009, oat grain from just before it was harvested, and roots from midsummer of 2009, at which point corn, soy and oat roots were expected to be around their maximum.

Soil samples taken in spring 2009 and 2010 were used in order to fulfill our first objective, to quantify the effects of cropping system and component practices on overall system soil physical, chemical and biological indicators and plant productivity. By considering two dates we were able to ensure that observed differences were not merely plot effects. Soil samples taken in spring, summer and fall of 2009 were used to track temporal changes in the soil and to test hypotheses about the temporal or spatial patterns of addition or disturbance associated with particular practices or crops. By observing seasonal dynamics, we were able to show the duration of effects, and were able to ensure that observed patterns were true over the whole growing season and not just spring.

Site description

The Marsden Farm Cropping Systems Experiment is a randomized complete block experiment with four replications located in Iowa Boone County, Iowa (42°01’ N; 93°47’ W; 333m above sea level). Average annual precipitation is 723 mm (Liebman et al., 2008), although in 2008, the site received 1100mm (National Weather Service, http://www.ncdc.noaa.gov) which qualified as enough rain to cause a 100 year flood. Soils vary across the experimental site and consist of approximately equal proportions of Clarion loam (fine-loamy, mixed, superactive, mesic, Typic Hapludolls, 2-5% slope), Nicolet loam (fine-loamy, mixed, superactive, mesic, Aquic Hapludolls, 1-3% slope) and Webster silty clay loam (fine-loamy, mixed, superactive, mesic, Typic Endoaquolls, 0-2% slope) (Liebman et al., 2008). Details on site history and management are found in Liebman et al., 2008 and Cruse et al., 2010.

Each of four blocks are separated by a 15.2 m grass border and divided into nine 85x18 m strips such that each crop in each rotation was represented once in each block in each year (Table 1). The soil is chisel-plowed to 15 cm in the fall after corn harvest and urea is spread in the spring before corn planting at a rate of 100 kg N ha $^{-1}$. In the3-yr rotation corn and soybean were followed by a year of small grains (triticale (× *Triticosecale* Wittmack) until 2005 but subsequently oat (*Avena sativa* L.) inter-seeded with red clover. The oat crop is harvested for grain, the straw is baled and taken off, and in the fall the red clover is moldboard plowed to 20 cm as a green manure along with 15.7 Mg ha^{-1} (fresh weight basis) composted beef cattle (*Bos taurus*) manure containing on average 128 kg N ha^{-1}, to provide nutrition for corn the following year. The 4-yr rotation consists of corn and soybean followed by a year of oats and alfalfa. In the third year oats are harvested as in the 3-yr rotation and the alfalfa is mowed once and continues on to a second year. The second-year alfalfa is harvested for hay three or four times, and plowed under as green manure in November along with composted manure, as in the 3-yr rotation. As moldboard plowing is always done in conjunction with manure addition, their effects are confounded. For corn in all systems, additional inorganic N is side-dressed in early June if needed in response to a late spring soil nitrate test taken to 30 cm. Lower thresholds are used in the conventional rotation. Side-dress N ranged from 0 kg N ha^{-1} to a high of 100 kg N ha^{-1} in 2008, the flood year. Thresholds used vary but are higher in the LEI rotations than in the conventional. Both P and K are periodically applied in all rotations as needed in response to standard soil tests.

Plant sampling and analysis

To quantify crop C input to the soil and to evaluate relative crop growth, harvested portions and aboveground stover were obtained from all crops in 2009. In fall directly before soybean harvest and after corn had dried down, corn grain and stover were collected in fall from four representative corn plants near the center of the field, and soybean grain and stalks, pods and leaves were sampled from two 0.5806 m^2 sample areas near the north and south ends of all plots. In October before plow-down, aboveground biomass samples of alfalfa and red clover were taken from four 0.25 m^2 sample areas. Plant samples were dried at 40^o C and ground before total C and N were determined by dry combustion. Grain removed from the corn and soybean crops was dried and weighed. Grain C and N were not measured, but were estimated using values from related hybrids grown on neighboring research plots. Values for manure weight, C and N from 2008 were also obtained from the Marsden field staff.

C inputs for each plot in crop (Y) were calculated as C as:

$$Total\ C\ inputs = \sum \{(aboveground\ residues\ from\ crop\ Y-1) + (\ belowground\ fall\ biomass\ from\ crop\ Y-1) + (composted\ manure) + (estimated\ exudates\ from\ crop\ Y)\} * (number\ crops\ in\ rotation)^{-1}$$

To estimate the distribution of inputs within the soil we assumed that moldboard plowing distributed 50% of the residues in the 0-10cm depth and 50% of the residues in the 10-20cm depth. This is consistent with ^{13}C labeling studies of residues buried by moldboard plowing conducted by Wander and Yang (2000). Some studies show up to 80% of residues being deposited in the 10-20cm depth after moldboard plowing (i.e. Allmaras et al. 1996), but equal mixing was assumed as being the most conservative for comparisons between chiseled and moldboard plowed systems.

To derive N fixation estimates, we obtained data from the field staff on biomass and N content of the harvested alfalfa. As the flooding in 2008 resulted in a poor stand of alfalfa in the second-year alfalfa plots, we used average biomass and N measurements from 2002-2008 to estimate typical removal rates.   For leguminous crops we used conservative estimates (N-fixed= 41% of alfalfa or red clover aboveground biomass N and 41% soybean grain N) from a recent study estimating N-fixation in the Mississippi valley (Russelle and Birr 2004).   We calculated average annual external input N as:

$$N\ inputs = \frac{\sum(Urea\ or\ compost\ N_{corn}),(estimated\ biological\ fixation\ N_{legumes})}{number\ of\ crop\ years}$$

A weakness of this method is that N-fixation rates are highly variable even within the same climate, and are likely to differ by system. The 4-yr rotation would be most affected by errors, in which three out of the four crop years involve N inputs by fixation.

Root sampling and analysis

To evaluate the influence of root inputs we measured root growth parameters—root length, diameter and quality— that should be directly affected by changes in soil and have the potential to alter the environment by direct additions and through indirect effects on structure. That is, soil and crop characteristics were not only measured as indicators of system function, but also as potential mechanistic actors.

Composite samples of four soil cores (5.08 cm diameter) were obtained from 0-10 and 10-20 cm depths. Cores were taken from between crop-row position in the oat and alfalfa plots and 15 cm from the crop-row in the corn and soybean plots. Samples from soil under oat were taken in late June just before the oat harvest. For all other crops, cores were taken in late July.   Cores from all crops were re-taken in late September in a similar manner. Root cores were put on ice after collection and kept at 4^{o} C until analysis within a month from the time of collection. For both sampling dates, root length, diameter and C and N content were determined. Root samples were elutriated to separate all low-density material from soil, and non-root debris was identified and discarded. Clean root samples were stored in 50% ethanol at 4^{o} C. Root length and diameter were measured on an Epson 1680 scanner (Epson

America Inc., Long Beach, CA, USA) utilizing WinRhizo root scanning software (WinRhizo, Regent Instruments, Québec, Canada). After scanning, root samples were dried, weighed, and ground to powder for C and N analysis by dry combustion on a Costech Elemental Analyzer (Model ECS 4010, Costech Analytical Technologies, Inc. Valencia, CA). Root exudates were estimated as 0.65*measured summer root C, after Bolinder et al. (2007). Results were used to estimate root length and root biomass. This method, while sufficient for comparing average root length and diameter between crops and rotations, likely underestimates root C. Extrapolation to field scale estimates will therefore be lower than actual values, particularly for tap-rooted plants. The influence of this underestimation of root inputs would be worst in the 4-yr rotation, in which three of the four crop-years included tap-rooted species.

Soil sampling and analysis

We wished to examine if soil quality, ability to retain organic matter and system efficiency were improved under the low-input rotations, particularly towards the end of the rotation under the hay crops. To quantify management effects on the soil we measured a variety of physical, chemical and biological soil quality parameters: bulk density (BD), water filled pore space (WFPS), whole soil C and N, particulate organic matter C (POM-C), potentially mineralizable N (PMN), and fluorescein diacetate hydrolysis rate (FDA). These assays have been shown to be sensitive to management (Schnurer and Rosswall, 1982; Franzluebbers and Arshad, 1996; Marriott and Wander, 2006; Karlen et al., 2006) and soil function (Wander, 2004). Wet aggregate stability and water filled pore space are measures of physical quality that are related to soil's ability to protect SOC from decay. The WFPS has been shown to correlate well with OM mineralization across a wide range of soils (Linn and Doran, 1984) and to be sensitive to tillage (Franzluebbers and Arshad, 1996), while BD is related to the plant roots' ability to penetrate the soil. We took soil samples from 0-10cm and 10-20cm depths to document different patterns of residue distribution and the associated variation in soil quality that were expected to result (Wander and Yang, 2000).

Soil cores from 0-20cm were obtained from each plot four times in 2009-2010 with a 5.08 cm diameter splittable soil probe. Two cores each were taken 10m from the north and south ends of each plot and composited to obtain a representative sample. All the corn, oat and alfalfa plots were sampled in April 2009 after spring soil disturbance; this was directly after corn planting and a few weeks after oat and alfalfa planting. Soybean plots were sampled in early May, directly after planting. All plots were sampled late July 2009 at approximately the time of maximum corn and soybean root development, and again in late September 2009, just before soybean harvest and well before any fall tillage. In April and May 2010, cores were taken as in 2009. For all sampling dates, cores were divided in the field into 0-10cm and 10-20cm segments, placed on ice for transport to the lab and kept at 4^{o}C.

Physical analyses

Within seven days of collection, cores for soil analysis were weighed for bulk density and sieved while fresh through an 8mm sieve. Next, 10-20g of fresh soil was weighed and oven-dried for 24 hr. at 105^{o}C to determine moisture content. Bulk density was calculated as oven-dried soil weight corrected for rocks and large residues. All soil chemical, physical and biological analyses described below were corrected for bulk density and reported on a volume basis. Water-filled pore space (WFPS) was calculated as:

$$WFPS = (field\ moisture) * (bulk\ density)/(1 - bulk\ density/2.65)$$

From a portion of air-dried <8mm soil all visible residues >1 mm were handpicked out with tweezers to yield a soil fraction to be used to measure structural, chemical and physical characteristics. A portion was dry sieved between 2mm and 1mm sieves and the resulting 1-2mm fraction was reserved for water-stable aggregate analysis.   The percentage of soil contained in water-stable aggregates (%WSA) were measured on air-dried soils from 2009 and 2010 using the standard wet sieving method adapted from Kemper and Roseneau (1986). Briefly, 10g of dry aggregates between 1-2mm in diameter were poured in a single layer on top of a 250 μm sieve, which was in turn placed on a 53μm sieve. Aggregates were slaked by just submerging them in water for five minutes, after which the sieves were placed on a machine which moved them up and down through a column of water at a constant rate for ten minutes. After sieving, material on top of each sieve was quantitatively washed into a 53μm nylon mesh, secured with a clip, dried at 60^{o}C for 24 hr., and weighed. From each aggregate size fraction, the sand and POM were extracted by quantitatively transferring sieved material into 40 mL Nalgene bottles and dispersing, drying and weighing the <53μm fraction as described for POM below. Sand-free macroaggregate and microaggregate concentrations were calculated as the weight of the aggregate fraction less the weight of the sand and POM fraction left following dispersion. As the macroaggregate fraction was more meaningful to our analyses, "%WSA" used henceforth refers to the macroaggregate fraction.

Soil texture was analyzed on soils from the summer of 2009 only, using the standard hydrometer method (Gee and Bauder, 1986).

Chemical and biological analyses

The remaining soil was mechanically crushed to pass through a 2mm sieve for use in total soil carbon (C) and nitrogen (N), particulate organic matter C and N (POM-C and POM-N), fluorescein diacetate hydrolysis, (FDA, a measure of heterotrophic microbial activity) and potentially mineralizable nitrogen (PMN) determination. In fall 2009 only, soils were finely ground and analyzed for total C and N by dry combustion. Organic C was obtained by subtracting inorganic C values determined for each plot after Bundy and Bremner (1972). Soil POM-C and N (concentration of organic C and N in the size fraction between 53μm and 1000μm) concentration was determined as described by Marriott and Wander (2006). The POM-C fraction was determined by dry combustion. The POM fraction as found to contain carbonates and thus, we developed appropriate correction factors by removing carbonates by fumigation as described by Harris et al. (2001) using soils collected in spring 2010. These correction factors were based on difference where the percent of C in carbonates was assumed to equal C in the uncorrected minus the corrected samples and applied to other dates. Soil PMN was determined with an anaerobic incubation, as described by Drinkwater et al. (1996) with some modifications. Three sets of 5g samples of air-dried soil ground to pass through a 2mm sieve were placed in 50mL centrifuge tubes. Next, 50mL 2 mol L^{-1} KCl were added to one set to quantify the amount of NH_4-N originally present in the soil. Samples were shaken for 1 hr. on a reciprocal shaker and vacuum-filtered through Whatman No. 42 filter paper, and analyzed colorometrically for NH_4-N after Sims et al. (1995). To the other two replicate sets, 10mL deionized water was added and incubated for 7 days at 40^{o} C. After one week, samples were mixed with 40 mL 2.5 mol L^{-1} KCl solution, shaken, before the solution was filtered and analyzed as in the first set of samples.

Enzyme activity was determined by the fluorescein diacetate (FDA) hydrolysis method, which represents microbial enzymatic activity, modified from that proposed by Schnurer and Rosswall (1982) as improved by Adam and Duncan (2001). Duplicate 1.00 g air-dried <2 mm soil samples placed in 40mL Nalgene centrifuge tubes and shaken with 20mL phosphate buffer (60 mmol L^{-1}, pH 7.6) and either 0.50ml FDA solution (4.8 mmol L^{-1}) or 0.50mL acetone in tightly capped bottles at 200 RPM on an orbital shaker at 23-24^{o} C. After 5 hr. samples were removed from the shaker and the reaction was stopped by the addition of 20mL acetone. Samples were centrifuged for six minutes at an angular velocity of 7000 RPM and extracted through Whatman No. 42 filter paper. Color development in the filtrate was determined via spectrophotometer at 490 nm against a standard curve. Soil FDA hydrolysis was calculated as the concentration in the FDA addition samples less the concentration in the acetone blanks, expressed as mg FDA hydrolyzed per g of soil and per hour of incubation.

Statistical analyses

To test the hypothesis that improvements in soil quality indicators and plant productivity would be driven by the amount and placement of organic residues, plant and soil factors and input C were compared between rotations.

Differences between rotations were analyzed as a randomized complete block design with four replications using PROC MIXED in the SAS system (SAS Institute, Cary, NC). In order to compare rotations under the same crop, we regarded each crop within each rotation as a treatment, giving the class variable crop(system) with nine levels. Other class variables were block, depth and year when appropriate. Block and its interactions run as random variables and the others as fixed variables. Rotation comparisons were made with estimate statements between the average of all crops in one system and the average of all crops in the other. All soil variables except total soil organic C (SOC) were analyzed using data from spring 2009 and 2010. Total SOC and C and N inputs were analyzed using fall 2009 data. Root length, diameter and quality were compared using data from summer 2009. Percentage of clay particles (<2μm) were used as a covariate when necessary, as the blocking did not entirely remove texture differences between rotations. Covariate interactions were removed from the model if they were insignificant at $p>0.05$ and class variable interactions were removed if they were insignificant at $p>0.35$. Data were transformed when necessary to meet assumptions of residual normality and homogeneity of variance. The square root transformation was performed on the POM-C, PMN, FDA and root length variables, and the natural log transformation on the stratification ratios for POM-C, PMN, FDA, and %WSA. Reported values are back-transformed LSMEANS, and rotation means for transformed variables are averages of back-transformed crop(system) LSMEANS. Differences in means were assessed using Tukey's test, and were considered to be significant at $p<0.05$ and marginally significant at $p<0.10$. Differences in written contrasts were assessed using a stepdown bonferroni adjustment. To explore the differential effect of rotations on depth distribution, for each soil and root variable we calculated a stratification ratio between the depths for each plot *i* as

$$ratio_i = \frac{value_i \ at \ 0-10cm}{value_i \ at \ 10-20cm}$$

The stratification ratios were analyzed as described above.

For our first hypothesis, a significant difference between sampling years was not considered to be important, particularly for the physical indicators, since samples were taken in different plots where texture is known to vary and under different climactic conditions. However, interactions with year were used to assess variability in crop(systems), depths, and crop(system) by depth interactions, and especially to determine if rankings and behavior patterns remained in the same despite the change of plot and climate.

To test our second hypothesis on the influences of specific crops on plant and soil response, we performed ANOVAs in PROC MIXED as before, using for the soil variables data from spring, summer and fall of 2009. Samples collected from the same plots on different dates were not analyzed as repeated measures, as the covariance structure met independence assumptions.

In order to evaluate the association of soil aggregation with other soil factors, we used the RSQUARE and CP model selection procedures in PROC REG of the SAS system to identify appropriate terms, and modeled them using PROC REG, using adjusted R^2 values to assess the percent of variability in %WSA that could be explained by the chosen factors. In order to evaluate the effect of soil physical structure on root growth, we regressed summer root length and average diameter against summer bulk density for each crop species using PROC REG in the SAS system. All assumptions were met.

Results

System inputs

Average yearly C inputs were significantly different in all three systems and ranked 3-yr>2-yr>4-yr (Table 2). The 0-10cm depth received significantly more input C than the 10-20cm depth in all rotations. Subsoil C additions were 3, 13, and 18% of total additions in the 2-yr, 4-yr and 3-yr rotations with the conservative assumption that moldboard plowing mixed residues equally between the depths. If, as has been suggested in some studies, up to 80% of moldboard plowed residue ends up below 10cm, up to 19% and 30% of the total C inputs in the 4-yr and 3-yr rotations may be added to the 10-20cm depth. The largest C inputs were to the soybean year (from the corn residues), while the second-year alfalfa stand had the lowest C inputs. The 3-yr and 2-yr rotations had similar average annual C input to the 0-10cm depth, while that of the 4-yr rotation received significantly lower C inputs than both. The average differences were largely attributable to differences in the frequency of corn in the rotation and the absence of surface residues derived from the alfalfa. In the 10-20cm depth input C to the 3-yr and 4-yr rotations was over 600% and 200%, respectively, that of the 2-yr rotation. This difference is due to the large inputs to the 10-20cm depth from moldboard plowing before the corn year. About 138.2 g C cm^{-3} was added as composted manure before corn in the 3-yr and 4-yr rotations, an annual average of 23g C m^{-2} and 17g C m^{-2} respectively, about 33% and 56% of the mean C inputs to their 10-20cm depths.. Estimated annual N inputs differed among all three rotations ($p<0.0001$), with the 3-yr and 4-yr rotations receiving 24% and 40% less external N respectively, than the 2-yr rotation for the 2009 season (Table 2).

Indicators of soil quality

Particle size analysis

Texture varied considerably across the experimental sites. Soils were loams and silty clay loams, with sand (53-2000μm), silt (20-53μm) and clay (<2μm) concentrations ranging from 21 to 46%, 30-47%

and 19-35%, respectively. The 10-20cm depth had more fine particles than the 0-10cm depth and the 3-yr and 4-yr rotations had a higher concentration of fines than the 2-yr rotation (data not shown). Texture was therefore used as a covariate for the other analyses.

Physical indicators: bulk density, percent water-filled pore space and wet aggregate stability

There were no system-based differences for any of the physical analyses when differences among rotations were made across both depths.

Spring bulk density varied between depths and years (Table 3), being higher in the 10-20cm depth than the 0-10cm depth. Bulk density tended to be low, between 0.8 and 1.2g cm^{-3} in the 0-10cm depth and between 0.9-1.3g cm^{-3} in the 10-20 cm depth, and did not differ between systems (Table 4). The lack of year interactions shows the results to be stable across both sampling years.

Crop(system), depth and year, and the interaction between crop(system) and depth were all strongly significant for spring WFPS (Table 3). The 10-20cm depth had higher WFPS than the 0-10cm depth. Overall the 2-yr rotation had a significantly higher WFPS than the 4-yr rotation, and marginally higher than the 3-yr; however, these differences were only significant in the 0-10cm depth (Table 4). Again, no year interaction was observed.

Spring %WSA showed significant differences between depths, the interaction between crop(system) and depth, year, and the interaction between depth and year. The 10-20cm depth had significantly higher %WSA than the 0-10cm depth. There was no overall difference between rotations, but at the 0-10cm depth the 3-yr rotation had lower %WSA than the 2-yr rotation (Table 4). Spring of 2010 had a greater %WSA than spring of 2009, but only in the 0-10 cm depth (data not shown).

Soil organic matter

When normalized for bulk density and calculated on a volume basis (mg SOM cm^{-3}), SOM stocks were found to be higher in the 10-20cm than the 0-10cm depth in all rotations (Table 4). However, when analyzed on a weight basis (g SOM g^{-1}) the 0-10cm depth had a higher concentration (data not shown). No system differences were observed at either depth. This is consistent with annual analyses done on soils taken from the 0-20cm depth since 2002, which show considerable yearly fluctuations but no significant overall change in SOM content (M. Liebman, personal communication, data not shown). As bulk density was not measured in these years, trends in SOM may be masked by changes in the effective depth of sampling.

Chemical and biological indicators: particulate organic matter C, potentially mineralizable N, enzymatic activity

Spring POM-C varied significantly by crop(system), depth, the interaction of crop(system) and depth, and the interaction of depth and year (Table 3). Averaged across both depths, both the LEI rotations had a higher mean POM-C than the 2-yr rotation (Table 4). This difference was a result of the strong stratification in the 2-yr rotation, which was absent in both LEI rotations (Table 4). The POM-C content of the 2-yr rotation, while not differing from the 3-yr rotation and higher than the 4-yr rotation in the 0-10cm depth, was greatly depleted in the 10-20cm depth compared to both LEI rotations (Table 4). On average, POM-C was greater in the 0-10cm depth than in the 10-20cm depth ; however, this difference was only significant in the 2-yr rotation. The POM-C was greater in the spring of 2009 than 2010, but only at the 0-10cm depth (data not shown).

Distribution of potentially available N mirrored that of POM-C. For PMN as well, depth and the interaction between depth and crop(system) were strongly significant due to the 2-yr rotations depletion in the 10-20cm depth (Table 3, 4). Overall, the 3-yr rotation had significantly and the 4-yr rotation marginally significantly greater PMN than the 2-yr rotation (Table 4). Unlike for POM-C, concentrations did not differ among the rotations at the 0-10cm depth.

In enzymatic activity (FDA) the strong effect of depth and interaction of depth with crop(system) was again present, along with a year effect (Table 3). This is not surprising given the different moisture conditions at time of sampling in the two years. While the 2-yr rotation had lower FDA that either of the LEI rotations in the 10-20cm depth, it was higher than either in the 0-10cm depth and there was no overall difference between the rotations. The FDA stratification ratio for the 2-yr rotation, while still significantly greater than for either of the LEI rotations, was less than for POM-C or PMN (Table 4)

Comparative plant response

Root parameters

Root parameters were analyzed from the midsummer sampling only. Summer RLD differed significantly by depth and the depth by crop(system) interaction (Table 3). Higher average RLD was observed in the 4-yr rotation than in the 2-yr rotation overall (Table 4). Again, values for the 2-yr rotation in the 10-20cm depth were significantly lower than those in the LEI rotations (Table 4). The stratification ratio in the 2-yr rotation was higher than in the 4-yr rotation, but not the 3-yr (Table 4).

Patterns in the corn roots paralleled the distribution of soil labile C, in that RLD in the 2-yr was more stratified than that observed in the 3-yr and 4-yr rotations (Figure 2). The same pattern was suggested in the soybean roots, but depth-based differences were not significant. Pre-planned contrasts to determine differences in corn RLD between rotations revealed the root length of 2-yr corn to be equal to that of the 3-yr corn but significantly less than that of 4-yr corn (Table 6). There were no rotation differences between soybean root lengths. Average RLD in the oats, alfalfa and red clover plots was higher than that in corn or soybeans (Table 6). While root C:N did not significantly differ by crop(system), contrasts revealed the root C:N ratio of the 4-yr soybeans to be significantly lower than that of the 2-yr soybeans (Table 7). Regression analysis revealed no linear relationship between bulk density and either RLD or average diameter (Table 6).

Corn and soybean productivity

Grain yields in 2009 in the LEI rotations were somewhat higher than those achieved in the 2-yr rotation (Table 5). Average 3-yr and 4-yr corn yields were 10% and 8% higher than conventional yields, respectively, while 3-yr and 4-yr soybean yields were 13% and 18% higher (Table 5). The difference was mildly significant in the 3-yr corn (p=0.09) and the 4-yr soybeans (p=0.09). These yields are consistent with those seen in previous years. Harvest index, calculated as grain dry weigh/total aboveground plant dry weight, did not differ among the rotations (data not shown).

Seasonal differences in soil quality

Soil physical indicators: bulk density, percent water-filled pore space and wet aggregate stability

Seasonal analysis of physical indicators—BD, WFPS and %WSA—showed strong depth-based differences and strong fluctuations through the growing season (Table 8). However, no crop or crop(system)-based differences were observed in any of the three indicators when averaged over all depths and seasons (Table 9). Values for all three measures observed in the 10-20cm depth were greater than those seen in the 0-10cm depth. Overall, BD and WFPS were highest in spring, declined in summer and rose again in fall. Conversely, %WSA rose from spring to summer, and declined sharply in fall.

While crop(systems) did not differ overall, variables behaved differently at different depths for all physical indicators (Figure 3). For corn and soybeans the 0-10cm depth had a higher BD and WFPS than the 10-20cm depth, but this difference was not significant for the oat, red clover or alfalfa. When depth differences in %WSA were compared for individual crop(systems), as well, aggregation in the 0-10cm depth was only significantly lower than that in the 10-20cm depth for the 3-yr and 4-yr soybeans.

For all three indicators, seasonal behavior differed at different depths. For BD, the increase from summer and fall was due to an increase the 10-20cm depth. Similarly the difference between spring and fall WFPS was observed in the 10-20cm depth but not the 0-10cm depth. An increase in %WSA from spring to summer was seen only in the 0-10cm depth.

For all three physical indicators, stratification ratios were more variable in the 3-yr rotation than in the other systems. In the 3-yr rotation, the ratio of BD, WFPS and %WSA in the surface to subsurface soil was consistently lower in the soybeans than in the oats or the corn (Table 9). This pattern was not observed in the other rotations. In the 4-yr rotation, WFPS was more stratified in the corn than in the oats or the alfalfa.

For WFPS a strong three-way interaction with crop(system), depth and season was observed. For all cropping phases besides the mixed oat and legume years in the 3-yr and 4-yr rotations, WFPS rose between summer and fall. However, in the oat and red clover phases WFPS did not rise again in fall, and in the oat and alfalfa phase it rose in fall but did not reach the same level it had in spring (Table 10). For both LEI systems, this was only true in the 10-20cm depth (data not shown).

Model selection procedures in PROC REG used to identify the soil variables that best predicted %WSA showed that in the 0-10cm depth enzymatic activity, whole soil N and clay were positively related to aggregate stability and that WFPS was negatively related (Table 11). At the lower depth, whole soil N and clay were again chosen; these variables and POM C:N were positively associated with stability at that depth. The model in the 10-20 cm depth accounted for more of the variability than was accounted for in the 0-10 cm depth (Table 11). When summer %WSA was modeled by itself, with root length as one of the variables given to the RSQUARE procedure, the procedure still identified textural variables as being important, along with a measure dynamic C—PMN or FDA.

Chemical and biological indicators: particulate organic matter C, potentially mineralizable N, enzymatic activity

All three chemical and biological indicators- POM-C, PMN and FDA- behaved similarly to each other and confirmed the patterns observed in the spring system comparisons. All three indicators strongly differed by depth and the interaction of crop(system) with depth (Table 8). Although for all indicators, overall means values in the 0-10 cm depth were higher than those found in the 10-20 cm depth, differences were only significant in the 2-yr corn and 2-yr soybeans. Even though not significant, the 4-yr corn and soybeans tended to have higher POM-C, PMN and FDA in the 10-20cm depth than in the surface depth (Figure 4).

Surprisingly, temporal sampling showed that POM-C, PMN and FDA did not differ between crops, and that the POM-C depth stratification ratios did not change with any of the crop phases of the rotations (Table 9). Soil POM-C levels did differ over the course of the growing season, and season interacted significantly with crop(system) (Table 8). Spring POM was marginally higher than fall POM, although the difference was only significant in the 3C crop(system) (Table 10). The difference between spring and fall was significant when averaged over all crops, but only at the 0-10cm depth (data not shown).

Neither PMN nor FDA differed by season or had any significant seasonal interactions. Average PMN was marginally higher under oat than under soybean (Table 9). Stratification ratios for PMN also remained stable over the course of the rotation in all rotations. For FDA, however, stratification ratios varied somewhat within the rotations. In the 2-yr rotation, the corn had marginally greater stratification than the soybeans. In the 3-yr rotation, the oats had marginally greater stratification than the soybeans, and in the 4-yr rotation, the alfalfa had marginally greater stratification than the corn (Table 9).

Discussion

Objective I

Our first objective was to quantify the effects of cropping system and component practices (tillage, residue and manure additions, and rotation length,) on cumulative changes in physical, chemical and biological indicators and plant productivity. Improvements in soil quality indicators and plant productivity were expected to be driven by the amount and placement of organic residues

The “year” variable showed the random error associated with climactic variables or input differences in a given year. Significant differences between spring of 2009 and 2010 were expected, as samples were taken at a different moisture and temperature, and had had different amounts of inputs. The insignificant crop(system) by year and crop(system)*depth*year interactions showed that the differences observed between crop(systems) and stratification patterns were a consistent characteristic of the rotations and conclusions were applicable to years beyond those sampled. The significant depth by year interaction, observed only in POM-C and %WSA, gave an indication both of how consistent observed differences between depths were and the relative variability of each depth.

Rotation effect on physical characteristics

Our hypothesis of improved soil physical quality under the LEI rotations was not supported. The fact that bulk density did not differ between rotations despite different tillage and organic input regimes suggests either that this measure was not very sensitive to management, or that damage due to tillage was offset by organic matter additions. Lal (1999) and Puget and Lal (2005) found similarly that tillage had no effect on bulk density in tillage experiments in Ohio, and Karlen (2006) noted that of soil quality indicators used to characterize different rotations in Wisconsin and Iowa, bulk density was the least sensitive. It also may be that in these very low bulk density soils, the range of bulk density is so small that changes are difficult to detect. Soil WFPS, conversely, was significantly higher in the 2-yr than in the

LEI rotations, a difference which came from the lower WFPS in the 0-10cm depth of LEI rotations. The tendency of less-tilled surface soil to retain a greater WFPS is well-documented (i.e. Linn and Doran, 1984; Hill et al., 1985; Franzluebbers and Arshad, 1996; Chavez et al., 2009), and may be due to a greater proportion of macropores in conventionally-tilled soils (Hill et al., 1985). The overall greater spring WFPS in the 2-yr rotation, particularly in the 0-10cm depth, suggests that this effect is occurring in the Marsden soils.

Contrary to what was expected, %WSA was not increased in the LEI rotations compared to the 2-yr rotation, despite their greater POM-C and root presence. Rather, %WSA differed mainly between depths and years like the other physical indicators. The decreased %WSA in the 0-10cm depth of the LEI rotations is likely associated with their more intrusive tillage, which has frequently been shown to damage soil surface structure (i.e. Liebig et al., 2004; Chung et al., 2008), and would, along with the decreased WFPS, indicate that tillage to some extent damaged the surface soil structure. This is especially true for the 3-yr rotation, which was the most frequently tilled. However, the 10-20cm depths were alike in all three rotations, suggesting the damage did not affect the subsurface, or was offset by the additional C inputs. The greater variability in bulk density, WFPS and %WSA associated with the top depth, coupled with the fact that they are consistently higher in the 10-20cm depth suggests that all three measures increased with lack of disturbance.

Rotation effect on chemical and biological characteristics

Overall, soil characteristics did not change in a manner that reflected the pattern of residue C inputs from the previous year. Although the 3-yr received significantly greater average annual additions to both depths, both LEI rotations behaved very similarly in terms of POM, PMN and FDA, which should be strongly influenced by organic matter. The 2-yr rotation received higher organic matter inputs than the 4-yr rotation, and yet was comparatively depleted in labile C. Volumetric SOM concentration did not differ between the rotations in spite of different levels of addition. More surprisingly, although the majority of the input C received by all rotations is in the top 10cm, even assuming unequal mixing, POM, PMN, and FDA concentrations were not higher at 0-10cm than at 10-20cm in the LEI rotations. In addition, SOM concentration was slightly higher in the 10-20cm depth, although this is probably largely a function of the higher bulk density in that depth.

When soil depth is considered there is some evidence that labile soil organic matter followed residue additions. The 2-yr rotation contained much less POM-C, PMN and FDA at the 10-20cm depth, which may reflect the very small amount of organic matter it received. Additionally, the 2-yr rotation received organic C exceeding the others in the 0-10cm depth, and at that depth had higher average spring POM-C than the 4-yr rotation and higher FDA than both. However, it was the enrichment of the subsoil in the LEI rotations relative to the 2-yr rotation in the indicators of biologically active C— POM-C, PMN, and FDA—that caused the LEI rotations to have greater active C despite the fact that relatively little of the total material was deposited at that depth. The significant interaction of depth with year, in which POM-C differed between years but only in the 0-10cm depth, suggests that like the physical indicators turnover and instability in the surface soil were greater than in the subsoil. Together, these factors suggest that the periodic distribution of material to the less disturbed, more highly structured bottom depth may play a role in the lack of stratification in the LEI soil biological and chemical indicators, and in their accumulation of POM-C relative to the 2-yr rotation.

Patterns in FDA distribution and abundance, showing it was stratified in the 2-yr rotation but with average concentrations similar to those found in LEI rotations, suggest that while enzymatic activity reflects the amount of labile C it is determined by other factors as well. Like POM-C and PMN, FDA was also stratified in the 2-yr rotation but less so. Since POM-C is very similar in all the rotations at the 0-10cm depth despite high addition rates to the 2-yr system one knows that decay rates must be greater in the 0-10cm soil. This is reflected by the enhanced C cycle enzymatic activity found in the surface depth of the 2-yr rotation and may be associated with the greater WFPS observed in that soil.

Rotation effect on plant response

Yield in the LEI corn and soybeans equaled or exceeded conventional yields on reduced external N, which suggests that system efficiency frequently observed under LEI rotations is indeed occurring in the Marsden sites. In studies on similar systems where increased efficiency has been observed, the effect has been attributed to increased fertilizer retention due to reduced macropore flow (compared to a conventionally fertilized no-till site) leading to greater nitrate retention (Nissen and Wander, 2003) and the coupled addition of C and N (Drinkwater et al., 1998), which directly boosts soil N sinks such as POM and water-stable macroaggregates (Nyiraneza et al., (2010).

While the rotations in our study did not differ in aggregate stability, or bulk density, they did differ in amount and distribution of POM and PMN and interestingly, had corn root distribution patterns which reflected this stratification. Roots in the 2-yr crops tended to cluster in the top 10cm, while roots of the LEI crops more uniformly colonized the top 20cm of the soil, particularly corn. Ball et al. (2005), reviewing root architectural responses to soil heterogeneity, cite mechanical impedance and non-uniform distribution of favorable growth conditions as dominant factors explaining differences in root behavior. In the Marsden soils, soil physical hardness and differences in nutrient distribution and water filled pore space were seen as likely combine to affect root growth. Despite their low bulk density, the soils at Marsden tend to set very hard, and physical impedance was seen as a possible reason why the 2-yr corn root length density should be so much higher in the 0-10cm depth. Failure to observe any relationship between bulk density and average root diameter or root length suggests impedance is not limiting root growth (Barber, 1995; Ball et al., 2005). Patterns in WFPS may better explain differences seen in corn root growth. Overall, WFPS was higher in the 0-10cm depth under 2-yr corn than under the

LEI corn, and stratification in WFPS was significantly stronger in the 3-yr corn than the 2-yr (though the 4-yr was not). It is possible that greater WFPS in the 0-10cm depth contributed to root concentration in the surface depth under the 2-yr corn. In the 3-yr and to a lesser degree the 4-yr corn, roots penetrated more deeply where the WFPS ratio in the 0-10cm:10-20cm was lower.

The presence of larger reserves of labile organic matter (POM-C, PMN and FDA) in the LEI subsoils and associated mineralization may have also provided an incentive for root proliferation below 10cm. While we are unaware of any studies which have compared belowground characteristics to determine if increased N use efficiency is linked with changes in root length or distribution, the literature suggests this may be a possibility. Roots are known to proliferate in areas of greater nutrient concentration (Fageria and Moreira, 2011), and deeper-rooted crops exploit more of the soil's available N (Thurup-Kristensen et al., 2003), suggesting a more efficient use of available N by roots which more uniformly explore the soil. Overall, the surface-applied urea, the high spring WFPS and the generally higher FDA hydrolysis rate observed in the top0-10 cm of the 2-yr plots suggest a richer mineralization environment was present at that depth, thereby giving little incentive for their roots to explore the lower 10-20cm. The idea that increased nutrition in the LEI rotations, as evinced by higher POM-C and PMN, may be responsible for the observed higher yields is supported by the findings of Spargo et al. (2011), who in a 13-year study comparing PMN under different rotations found a strong linear relationship between corn yields and soil PMN. Concentration of roots in a smaller area may hurt plant health, by leading to localized depletions in water or nutrients, as well as providing better conditions for the spread of disease (Ball et al., 2005). The concentration of the 2-yr corn roots in the 0-10cm depth was the main difference seen between the LEI and 2-yr corn, suggesting that this may be a reason why corn in the LEI rotations produced greater yield on less external N inputs.

Possible differences in location of soil mineralizable N in the soybean phase are especially suggestive as experiments done on deep placement of slow-release fertilizer in soybean show that while mineral N in the surface lowers soybean N-fixation, mineral N placed below the nodulation zone does not (Takahashi et al., 1991; Salvagiotti et al., 2009). Enhanced N-supply derived from N fixation in the 0-10cm depth and from SOM mineralization in the 10-20cm depth combine to explain why soybean biomass and yield are higher in the LEI than 2-yr systems. This idea is supported by the lower C:N ratio of the LEI soybean roots.

For all physical, chemical and biological indicators, as well as plant response, the 3-yr and 4-yr rotations had behaved very similarly. There was no obvious response to increased rotation length or crop diversity between the two. However, as the plots were only eight years old at time of sampling, differences may not have had time to manifest. These results are similar those seen on a thirteen-year old study in Maryland, in which corn yields were found not to differ between an organic rotation receiving manure one out of every two years, and another, longer rotation which received manure two out of every six years (Spargo et al., 2011), although both differed substantially from a conventional rotation. In addition, similarly to our study, potentially mineralizable N and labile C did not improve with the longer diversified rotation.

Objective II

To meet our second objective we examined temporal responses to events within the rotations to help us determine the effect of specific practices on plant and soil response. We used the combination of spring, summer and fall 2009 measurements to determine how particular crops were affected by the previous crop or management, and the legacy they left to the subsequent crop. If crop interacted significantly with the season, it provided information on how the indicator was changed during the season, and allowed us to link soil changes with crop growth or tillage events. In addition, our system comparisons were done only on soils collected in spring. By comparing patterns seen in our first objective with those seen in soils taken over the course of the growing season, we were able to validate if an effect was only seen in spring (short-term, seasonal effect) or characterized the whole season as a system effect.

Temporal response of physical indicators

Bulk density's strong seasonal variation and lack of interaction between crop(system) and season confirm that it was relatively insensitive to management. It is surprising that there were no differences in bulk density of plots during the corn phase of the rotation, as LEI rotations that had been moldboard ploughed the previous fall, and the 2-yr corn plots had had no tillage. However, the fact that bulk density in the 0-10cm depth was significantly less than that of the 10-20cm depth only in corn and soybean phases suggest that 0-10cm soils may have been slightly loosened by tillage and the addition of organic matter, and hardened over time under the oats, red clover and alfalfa. Franzluebbers et al. (1995) also found tillage to have a loosening effect on bulk density which quickly disappeared.   Work by Hill et al. (1985), also on central Iowa silt loams, suggested that densification in these soils was primarily due to loss of pores >150μm. They concluded that conventionally tilled soils were more prone to densification than no-till soils, due to their larger proportion of macropores. Soil WFPS followed the same seasonal pattern as bulk density, partially because bulk density was used to calculate it.   However, unlike bulk density, WFPS does behave differently in different crop(systems) at different dates. The WFPS in the 10-20cm depth of the hay crops did not rise in fall to the same extent that it did under corn and soybeans, reflecting that they had plants actively growing throughout the season. Hence, the subsoil in the 3-yr and 4-yr rotations were drier for longer than those in the 2-yr rotations, and moisture conditions were less stratified in the LEI crops for a greater proportion of the time. If the 10-20cm depths in the LEI rotations had longer periods of dryness than in the 2-yr rotation, it may help explain their relative enrichment in organic matter. Franzluebbers and Arshad (1996), working on heavy soils in Canada, observed that organic matter in the surface depth of a no-till soil was more decayed than in the surface depth of a tilled soil. They attributed this effect to the higher WFPS they had also observed in the soil surface. Linn and Doran (1984), working across a wide range of soil types, found that %WFPS consistently predicted rate of OM mineralization, and that mineralization tended to peak at 60% WFPS before oxygen became limiting. The 2-yr rotation's average WFPS of 55% at the lower depth suggests that it may have been at around this maximum value.

Similarly to the other physical indicators, %WSA differed strongly between depths and seasons, rather than between crop(systems). This was contrary to our hypothesis, which predicted that %WSA would increase under the hay crops and be higher in the LEI rotation as a whole, serving to protect OM. While bulk density and WFPS were higher in spring and fall and lowest in summer, %WSA peaked in summer, particularly in the 0-10cm depth. Earlier studies have demonstrated aggregation is promoted by the drying action of roots (Hallett et al., 2009), which were at their maximum in the 0-10cm depth in summer. It seems likely that the presence of actively growing plants dried the soil, decreased bulk density, and increased aggregation in summer, while the root senescence and decay and the associated soil rewetting contributed to the steep aggregate decline in fall. Model selection procedures in PROG REG do suggest that aggregation is inversely related to WFPS in the top depth; however, in both depths texture and whole soil OM were much stronger predictors of aggregate stability. Summer %WSA correlated with neither summer root length nor WFPS. The strong relationship observed between %WSA, soil texture and SOM suggests that %WSA at Marsden is primarily a function of seasonal soil water interacting with soil characteristics that are only slowly, affected by management if at all. This is especially true in the 10-20cm depth

The LEI soybeans, and particularly those in the 3-yr rotation, appeared to suffer some surface structural degradation from the previous soil treatment. In general, a high stratification ratio in both physical and chemical indicators is thought to be beneficial to the soil, indication good erosion control, infiltration and nutrient storage capacity (Franzluebbers, 2002). All physical indicators in the 3-yr soybeans had a lower ratio of 0-10cm: 10-20cm than either of the other crops in the rotation; it was the only rotation in which different crops had significantly different stratification ratios. While the differences are not significant between the cropping phases at the same depth (data not shown), the decrease in stratification of physical indicators over the course of the LEI rotations does appear to be due to recovery at the top, rather than degradation at the bottom. For most soils, inversion tillage has been shown to damage soil structure in the topsoil by physically breaking it, and by causing mineralization of organic matter (Franzluebbers et al., 1998). As the 3-yr rotation was also the most tilled, the relative degradation of physical indicators in the surface to the subsurface may be evidence of structural degradation. Puget and Drinkwater's work (2005) indicates that the roots of tilled-in green manures strengthen soil aggregates of the following crop. This effect would explain why damage from tillage would not necessarily be seen in corn, the year directly following tillage, but rather in soybean, at which point the aggregative effect of the roots would not be likely to be operative (Abiven et al., 2009). In this case, the decreased stratification in the oat/ red clover physical indicators could indeed be evidence that the oat and red clover roots do indeed improve soil structure in the damaged 0-10cm depth.

Temporal response of biological and chemical indicators

Contrary to our hypothesis that improved soil quality would be observed under the oat, red clover and alfalfa, POM-C, PMN and FDA were not increased during or after these crop phases. All indicators remained surprisingly stable over the course of the season and the rotation, considering the different inputs, levels of tillage and crops grown. The strong, stable crop(system) by depth interaction in POM-C,PMN and FDA, which did not vary with season, confirmed that the effect seen in the spring comparisons was consistent throughout the growing season. The only seasonal interaction observed was when the 3-yr corn declined in POM-C from spring to fall. The observable decline in POM-C and

PMN from their high spring level suggest that POM derived from the red clover residue is more labile than that of other inputs, particularly as the decline is similar at both depths. For both POM-C and PMN, in the spring comparisons, the 3-yr corn was higher than the 2-yr corn. Furthermore, when both spring 2009 and 2010 were analyzed together (Table 3), a significant crop(system) effect in POM-C and a marginally significant one in PMN were both due to the 3-yr corn having a higher concentration than the 2-yr corn. However, when spring was averaged with summer and fall, the difference disappeared. Fifty nine percent of the organic inputs to the 3-yr corn were from the red clover biomass, which is expected to be very labile (Johnson et al., 2007; Abiven et al., 2009). The average of all crops shows a decline in POM-C from spring to fall only at the 0-10cm depth, suggesting the decay environment overall is more active at the top depth.

An explanation for the faster decay environment in the 0-10cm depth is that as there are more water-stable aggregates 10-20cm depth which is less-disturbed; material which enters that depth has a better chance of being protected by association within an aggregate. While aggregates are known to be a major sink of fertilizer N (Aoyama et al., 1999; Nyiraneza et al., 2010) and to be important in organic matter protection (Six et al., 2002), they are also very susceptible to disruption (Nyiraneza et al., 2010) making them very transient sinks. Many tillage studies have shown that the less disturbance from management that occurs, the more aggregates are observed and the stronger the soil's protective capacity is (i.e. Douglas and Goss, 1981; Smettem et al., 1992; Chen et al., 2009). So if, as the evidence suggests, disturbance is less in the 10-20 cm depth of the soil, protection of organic matter by aggregation may still be an important mechanism for preserving OM in the LEI rotations although %WSA was mostly depended on non-management factors. If this mechanism is occurring, LEI management may help conserve organic matter by moldboard plowing more of it to where it is more protected.

The 4-yr rotation's higher proportion of roots and compost returned to the soil may help explain why the 3-yr and 4-yr rotations have very similar amounts of POM-C and PMN despite their widely different organic matter inputs. The 3-yr corn and 4yr-corn plots both received a large amount of fresh, N-rich material but the temporal decline in POM-C was observed only in the 3yr-corn plots. The difference in response may be due to the fact that composted manure accounted for 59% of the C added to the 4-yr system and only 36% of the C added to the 3-yr phase. As compost is a more resistant organic material than the clover residue its contribution to POM-C is longer lasting in these soils. This is consistent with other works that have shown that composted manure is a highly recalcitrant material with long lasting benefits to soil (i.e. Sainju et al., 2008; Abiven et al., 2009; Nyiraneza et al., 2010). In addition, the 4-yr rotation returns a higher proportion of root material, which has also been suggested to be recalcitrant and have a slower turnover rate than shoot-derived material (Puget and Drinkwater,2001; Rasse et al., 2005).

The idea of a more active decay environment in the 0-10cm, and a base of resistant POM-C in the 10-20cm depth of the LEI rotations may explain the surprising result that despite the large input of corn residue to the surface the preceding fall, no pulse in POM was seen in any of the rotations during the soybean year, and stratification in POM-C, PMN and FDA did not increase from the corn year to the soybean year. This effect would be explained if the high surface nitrogen and WFPS in 2-yr rotation and disturbance in the LEI rotation caused the bulk of surface-added residues to be quickly mineralized, while the lack of the disturbance in the 2-yr 10-20cm depth and the large proportion of resistant material in the LEI 10-20 cm depth created a stable pool of POM-C which changed relatively little between crops or seasons.

Conclusions

This study aimed to identify specific links between management practices, soil quality and crop performance in the Marsden plots in order to account for increased corn and soybean yields observed in the LEI rotations. We conclude that the deep tillage to incorporate manure is likely responsible for creating a large, stable pool of biologically active C in the subsoil of the LEI rotations that persists though all cropping phases. Because of this accumulation, the LEI rotations stored overall more POM-C and PMN than the 2-yr rotation. The more uniform distribution of labile material coincides with greater root exploration of the soil profile in the LEI corn and to some extent soybeans. Relationships between bulk density and root length and diameter did not suggest that physical impedance was a barrier to root growth in these soils. The differential distribution of water and nutrients may better explain differences in crop growth. Soil properties and productivity in the two LEI rotations did not differ, suggesting that rotation length and overall organic inputs were less important to differences seen in soil quality and crop growth than the type and degree of incorporation of organic matter. However, as the eight-year period of our study included only two complete cycles of either rotation for most plots, these subtler differences may become apparent with time.

Contrary to our hypotheses, in this high clay and organic matter soil aggregation was not increased by the cover crop root systems, but rather depended on stable soil properties such texture and clay, and was only clearly affected by season, probably due to interactions with root growth, changes in soil water. We found some evidence that the greater tillage in the 3-yr rotation may have decreased %WSA in the 0-10cm depth; however, increases under oats appear to have offset this difference and overall %WSA was not decreased relative to the other rotations.

The fine-textured, high organic matter soils at our study site represent some of the most agriculturally important soils in the Midwest. Our study demonstrated that in these soils high productivity and good soil quality can be achieved using LEI practices and suggest that the periodic deep incorporation of manure is particularly advantageous. Further work will explore how organic matter type, placement and interaction with soil physical structure contributed to the differences observed between the rotations.

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Table 1. Summary of cropping system management. Experiment was laid out in a randomized complete block design with four replicates, with each crop phase in each system present in each block in each year.

Management practice	**Cropping systems**		
	2-yr	**3-yr**	**4-yr**
Crop rotation*	C-S	C-S-O/RC	C-S-O/A-A
Primary tillage$	Ch	MP, Ch	MP, Ch
N fertility%	Urea	Compost	Compost

* *C* corn, *S* soybean, *O* oat, *RC* red clover, *A* alfalfa.

$ *Ch* chisel plow to 15 cm before soybean, *MP* moldboard plow to 20 cm before corn.

% *Urea* 100 kg N ha^{-1} as urea spread in spring before corn plus late spring sidedress, *Compost* around 128 kg N ha^{-1} as composted beef manure, incorporated in fall before corn.

Table 2. Estimated average of yearly carbon (C) and nitrogen (N) inputs to each crop phase. Aboveground biomass inputs to each crop represent the stover measure in fall 2009 from the crop preceding it in the rotation. Root biomass C represents the C measured in roots collected in fall 2009 from the crop that directly preceded in the rotation. Total C inputs are the sum of aboveground and root biomass C from the previous crop, manure C incorporated the previous fall (138.2g C cm^{-3}), if an and root exudate C calculated after Bolinder et al. (2007) as 0.65*summer root C. It was assumed that moldboard ploughing mixed organic matter equally between the 0-10 cm and 10-20 cm depths (Yang and Wander, 2000). Total N inputs were estimated as the sum of N applied as fertilizer or manure in 2008 and N-fixation estimated according to Russelle and Birr 2004 as 0.41*(stover N + root N + yield N)

Crop	**Rotation**	**Aboveground biomass C inputs**						**Root biomass C inputs** *g C m^{-2}*				**Total C inputs**						**External N inputs** *g N m^{-2}*	
		0-10cm		**10-20cm**		**0-20cm**		**0-10cm**	**10-20cm**	**0-20cm**		**0-10cm**		**10-20cm**		**0-20cm**		**0-20cm**	
Average of all crops	**2**	293.13	a	0.00	c	293.13	a	7.88	5.32	13.20		309.19	a	9.83	c	319.02	b	13.69	a
	3	266.87	b	38.07	a	304.93	a	5.96	5.26	11.22		302.07	a	70.41	a	372.47	a	10.37	b
	4	172.19	c	3.68	b	175.86	b	8.00	5.02	13.02		209.24	b	30.73	b	239.97	c	8.26	c
Corn	2	211.57	a	0.00	c	211.57	b	7.96	3.56	11.52		226.08	a	7.64	c	233.71	bc	16.80	a
	3	114.20	b	114.20	a	228.40	a	7.02	3.76	10.78		194.20	a	191.49	a	385.69	a	12.47	b
	4	14.71	c	14.71	b	29.42	c	12.44	6.27	18.71		101.86	b	97.93	b	199.79	c	12.47	b
	Mean					***156.46***	***C***			***13.67***	***A***					***273.06***	***B***	***13.91***	***A***
Soy	2	374.68		0.00		374.68		7.80	7.08	14.88		392.31		12.02		404.33		10.57	b
	3	428.00		0.00		428.00		4.58	7.80	12.38		441.82		12.14		453.96		12.09	ab
	4	413.56		0.00		413.56		6.17	6.23	12.40		446.21		10.93		457.14		12.84	a
	Mean					***405.41***	***A***			***13.22***	***A***					***438.48***	***A***	***11.84***	***B***
Oat/legume	3	258.40		0.00		*258.40*		6.27	4.21	10.49		270.19		7.58		277.77		6.54	a
	4	260.47		0.00		260.47		8.89	4.11	13.00		277.28		6.40		283.68		2.20	b
	Mean					***259.44***	***B***			***11.74***	***AB***					***280.72***	***B***	***4.37***	***D***
Alfalfa	4	0.00		0.00		0.00		4.49	3.47	7.96		11.61		7.68		19.28		5.52	
	Mean					***0.00***				***7.96***	***B***					***19.28***	***C***	***5.52***	***C***

Different lowercase letters represent differences between systems at a specified crop and depth that were significant at $p<0.05$. Different uppercase letters represent differences that were significant at $p<0.05$ between values for each crop averaged over all rotations and both depths.

Table 3. Analysis of variance of soil parameters and stratification ratios on soils from spring 2009 and 2010. Bulk density (BD), water-filled pore space (WFPS), percentage of water-stable macro-aggregates (%WSA), particulate organic matter C (POM-C), potentially mineralizable N (PMN) and enzymatic activity (FDA), were performed on soils collected in spring 2009 and spring 2010. Soil organic matter (SOM) was measured on soils collected in fall 2009 only. Root length diameter (RLD and root C to N ratio (Root C:N) are from root samples collected in summer of 2009 only, and soil organic matter (SOM) was measured on soils collected in fall 2009 only.

Source	**BD**	**WFPS**	**%WSA**	**SOM**	**POM-C**	**PMN**	**FDA**	**RLD**	**Root C:N**
	p-value								
Crop(system)	0.0608	<.0001	0.4608	0.6869	0.0298	0.0894	0.8815	0.1063	0.5133
Depth	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.1314
Crop(system)*Depth	0.1442	0.0027	0.014	0.2028	<.0001	<.0001	<.0001	0.0064	D
Year	0.0006	<.0001	0.0281	NA#	0.0763	0.4862	0.0008	NA	NA
Crop(system)*Year	D*	0.5748	0.3404	NA	0.0829	0.0938	0.949	NA	NA
Depth*Year	0.3829	0.4232	0.0421	NA	0.0393	D	0.2801	NA	NA
Crop(system)*Depth*Year	D	0.2399	0.1542	NA	0.2982	D	0.105	NA	NA
Clay	<.0001	D	<.0001	<.0001	0.0595	0.0062	0.0043	0.0385	D
	Stratification ratio								
	p-value								
Crop(system)	0.1925	<.0001	0.0609	0.2733	<.0001	0.0003	0.0003	0.0137	
Year	0.5867	0.0139	0.0277	NA	0.0493	D	0.0755	NA	
Crop(system)*Year	0.0986	0.0667	0.2212	NA	D	D	0.0431	NA	

*"D" signifies a term dropped from the model with a p-value >0.35 for class variables and >0.05 for the covariate. # "NA" signifies a term not included in the model.

Table 4. Soil variable means and stratification ratios for soils collected in spring 2009 and 2010 from 0-10cm and 10-20cm depths. Soil organic matter (SOM) was measured on soils collected fall 2009 and Root Length Density (RLD) on samples collected in summer of 2009. Overall system differences values are linear combinations of the LSMEANS of all crops within each rotation, over both dates and both depths. Rotation by depth means values are linear combinations of the LSMEANS of all crops within each rotation averaged over both dates but separated by depth.

Depth		*Mean values*															
		BD		**WFPS**		**%WSA**		**SOM**		**POM-C**		**PMN**		**FDA**		**RLD**	
	Rotation	*g cm^{-3}*		*%*		*%*		*mg cm^{-3}*		*mg C cm^{-3}*		*µg cm^{-3}*		*µg cm^{-3} min^{-1}*		*cm cm^{-3}*	
0-20cm	**2**	1.13	a	49.25	a	55.68	a	51.23	a	1.90	b	31.24	b	0.96	a	2.72	a
	3	1.09	a	46.50	ab	52.41	a	49.64	a	2.31	a	40.44	a	0.96	a	2.90	ab
	4	1.11	a	45.48	b	55.73	a	49.86	a	2.19	a	37.26	ab	0.92	a	3.41	b
0-10 cm	**2**	1.05	a	43.92	a	55.56	a	48.81	a	2.91	a*	44.46	a	1.29	a	3.35	
	3	1.00	a	37.83	b	46.66	a	45.18	a	2.50	a	42.05	a	1.00	b	3.23	
	4	1.04	a	37.80	b	52.32	a	46.93	a	2.35	a*	39.62	a	0.96	b	3.80	
10-20 cm	**2**	1.22	a	54.59	a	55.81	a	53.43	a	0.89	b	18.02	b	0.62	b	2.09	b
	3	1.17	a	55.17	a	58.16	a	51.53	a	2.12	a	38.82	a	0.92	a	2.57	ab
	4	1.17	a	53.16	a	59.14	a	54.45	a	2.03	a	34.90	a	0.88	a	3.03	a
0-10 cm	**mean**	1.03	b	39.85	b	51.52	b	46.97	b	2.59	a	42.04	a	1.08	a	3.46	a
10-20 cm	**mean**	1.18	a	54.30	a	57.70	a	53.14	a	1.68	b	30.58	b	0.81	b	2.56	b
		Stratification ratio															
	2	0.90	a	0.83	a	0.90	a*	0.87	a	3.34	a	2.40	a	1.85	a	1.71	a
	3	0.88	a	0.69	b	0.73	a*	0.86	a	1.09	b	1.00	b	1.06	b	1.40	ab
	4	0.90	a	0.72	b	0.86	a	0.86	a	1.18	b	1.14	b	1.08	b	1.30	b

Different lowercase letters represent significant differences between the systems at p<0.05 or significant differences between rotations at each depth at p<0.05, using a stepdown bonferroni adjustment for estimates. *Asterisks between the same letter refer to differences at p<0.1. System differences are obtained by contrast statements comparing means of all crops within the rotations.

Table 5. Corn and soybean aboveground biomass, mean yield, measured in Fall 2009. Values are LSMEANS of crop(systems).

Crop	Rotation	Biomass	Yield	
		Mg ha^{-1}		
Corn	**2**	8.4	11.4	a*
	3	9.6	12.6	a*
	4	9.3	12.4	a
Soybeans	**2**	5.0	2.9	a*
	3	6.0	3.3	a
	4	6.0	3.5	a*

Different lowercase letters represent significant differences between the crop(systems) at $p<0.05$ or significant differences between rotations at each depth at $p<0.05$, using a stepdown bonferroni adjustment for estimates. Asterisks between the same letter refer to differences at $p<0.1$

Table 6. Pre-planned contrasts for root-length density in summer, conducted as one-tailed t-tests. Coefficients of determination (R^2) for linear regression between summer corn root parameters (Root length density (RLD) and average diameter (AvgDiam)) and bulk density (BD). All assumptions were met.

Hypothesis	**t-value**	**pr>t**
2C < 3C	-0.48	0.32
2C < 4C	-1.77	0.04
Corn, soybean < oat, alfalfa and red clover	-2.75	0.01

Model	**Parameter Estimate**	**Adjusted R^2**	**pr>F**
RLD=BD	-3.27118	0.0733	0.1072
AvgDiam=BD	0.07493	0.0592	0.1321

Table 7. Means and differences in summer soybean root C:N ratios. Values are LSMEANS. Pre-planned contrasts for summer soybean root C:N, conducted as one-tailed t-tests.

Rotation	Depth	C:N
2	0	23.3
	10	22.3
3	0	17.7
	10	18.4
4	0	15.8
	10	15.5
Hypothesis		
3S<2S	1.03	0.16
4S<2S	1.64	0.06

Table 8. Analysis of variance of soil and root parameters and stratification ratios from the 2009 growing season. Particulate organic matter C (POM-C), potentially mineralizable N (PMN), enzymatic activity (FDA), percentage of water-stable macro-aggregates (%WSA), bulk density (BD) and water-filled pore space (WFPS) were performed on soils collected in spring, summer and fall of 2009.

	BD	**WFPS**	**%WSA**	**POM-C**	**PMN**	**FDA**
	p-value					
Crop(system)	0.2107	0.1861	0.4262	0.148	0.037	0.6237
Depth	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Crop(system)*Depth	0.0245	<.0001	0.0012	<.0001	<.0001	<.0001
Season	<.0001	<.0001	<.0001	0.0285	0.3173	0.3305
Crop(system)*Season	D	0.0049	D	0.0083	0.1875	D
Depth*Season	0.0004	0.0001	0.0543	0.0135	D	0.0665
Crop(system)*Depth*Season	D	<.0001	D	D	D	D
Clay	0.0002	D	<.0001	0.8867	0.0195	0.0056
	Stratification ratio					
	p-value					
Crop(system)	0.0585	0.0033	0.0082	<.0001	<.0001	<.0001
Date	0.0007	0.0489	0.0418	0.0027	0.2562	0.0994
Crop(system)*Date	D	<.0001	0.2755	D	D	D

Table 9. Soil variable means and stratification ratios for soils collected in the 2009 growing season from 0-10cm and 10-20cm depths. Soil organic matter (SOM) was measured on soils collected fall 2009. Overall system difference values are linear combinations of the LSMEANS of all crops within each rotation, over both dates and both depths. Rotation by depth mean values are linear combinations of the LSMEANS of all crops within each rotation averaged over both dates but separated by depth. Differences in depth stratification ratios between crop(systems) values are linear combinations of the LSMEANS of the stratification ratio

	BD		WFPS		%WSA		POM-C		PMN		FDA	
	g cm^{-3}		*%*		*%*		*mg cm^{-3}*		*µg cm^{-3}*		*µg cm^{-3} min^{-1}*	
Means												
Spring	1.13	a	48.84	a	52.47	b	2.22	a*	37.28	a	0.88	
Summer	1.02	b	33.02	b	58.33	a	2.10	a	36.26	a	0.82	
Fall	1.09	a	46.74	ab	42.39	c	1.99	a*	38.87	a	0.85	
Corn	1.07		42.59		50.71		2.05	a	36.12	a	0.83	
Soybean	1.07		43.27		50.65		2.08	a	35.01	a*	0.83	
Oat/legume	1.09		43.67		51.95		2.26	a	42.85	a*	0.89	
Alfalfa	1.13		40.87		51.54		2.07	a	39.26	a	0.80	
Stratification ratios												
2-Corn	0.87	a	0.80		1.04		3.40		2.25		2.29	a*
2-Soybean	0.88	a	0.84		0.94		2.34		2.25		1.57	a*
3-Corn	0.91	ab*	0.80	a	0.87	a	1.19		1.06		1.02	a
3-Soybean	0.79	b*	0.68	b	0.62	b	1.09		0.93		0.87	a*
3-Oat/RC	0.93	a	0.85	a	0.87	a	1.66		1.28		1.37	a*
4-Corn	0.87	***a***	0.68	b	0.91		0.94		0.97		0.85	a*
4-Soybean	0.87	a	0.79		0.73		0.87		0.91		0.92	a
4-Oat/Alfalfa	0.93	a	0.87	a	0.80		1.17		1.02		1.08	a
4-Alfalfa	0.96	***a***	0.91	a	0.80		1.47		1.24		1.31	a*

Different lowercase letters represent significant differences between the systems at p<0.05 or significant differences between rotations at each depth at p<0.05, taking into account experiment-wise error. System differences are obtained by contrast statements comparing means of all crops within the rotations. *Asterisks between the same letter represent significant differences at p<0.10

Table 10. Crop(system) by season interactions in water-filled pore space (WFPS) and particulate organic matter C (POM-C). Values are linear combinations of the LSMEANS of both depths for each crop(system) within each season.

	2-yr				3-yr						4-yr							
	Corn		**Soybean**		**Corn**		**Soybean**		**Oat/RC**		**Corn**		**Soybean**		**Oat/alf**		**Alfalfa**	
	WFPS (%)																	
Spring 2009	49.67	a	54.02	a	45.11	a	50.21	a	49.21	a	45.94	a	46.85	a	54.31	a	44.29	a
Summer 2009	34.26	b	33.07	b	32.78	b	29.78	b	36.87	b	31.76	b	31.57	b	33.91	c	33.16	b
Fall 2009	50.95	a	47.55	a	48.40	a	47.92	a	43.28	ab	44.48	a	48.47	a	44.46	b	45.17	a
	POM-C (g cm^{-3})																	
Spring 2009	1.81		1.93		2.79	a	2.28		2.14		2.68		2.14		2.44		1.87	
Summer 2009	1.71		1.95		2.22	ab*	2.22		2.24		2.19		2.24		2.22		1.97	
Fall 2009	1.44		2.16		1.48	b*	1.89		2.14		2.26		1.92		2.42		2.38	

Different lowercase letters represent significant differences between the seasons within each crop(system) at p<0.05

*Asterisks represent significant differences between the seasons within each crop(system) at p<0.1

Table 11: Multiple regression models for aggregate stability in spring, summer and fall of 2009 and spring of 2010. Parameters were identified using a combination of RSQUARE and CP procedures in PROC REG, and standardized parameter estimates were obtained through modeling the identified parameters in PROC REG.

Depth	Parameter	Standardized parameter estimate	Model adjusted R^2	Model Pr(>F)
		All seasons		
0-10 cm	FDA	0.17	0.37	<0.0001
	Whole Soil N	0.33		
	Clay	0.21		
	WFPS	-0.24		
10-20 cm	POM C:N	0.15	0.47	<0.0001
	Whole soil N	0.41		
	Clay	0.37		
0-20 cm	POM-N	-0.16	0.37	<0.0001
	Whole soil N	0.33		
	Clay	0.38		
		Summer 2009		
0-10 cm	PMN	0.14	0.36	0.0012
	Soil C:N	-0.19		
	Sand	-0.60		
10-20 cm	FDA	0.12	0.60	<.0001
	Sand	-0.52		
	Bulk Density	-0.35		
0-20 cm	FDA	0.17	0.45	<.0001
	Sand	-0.95		
	Silt	-0.38		

Figures

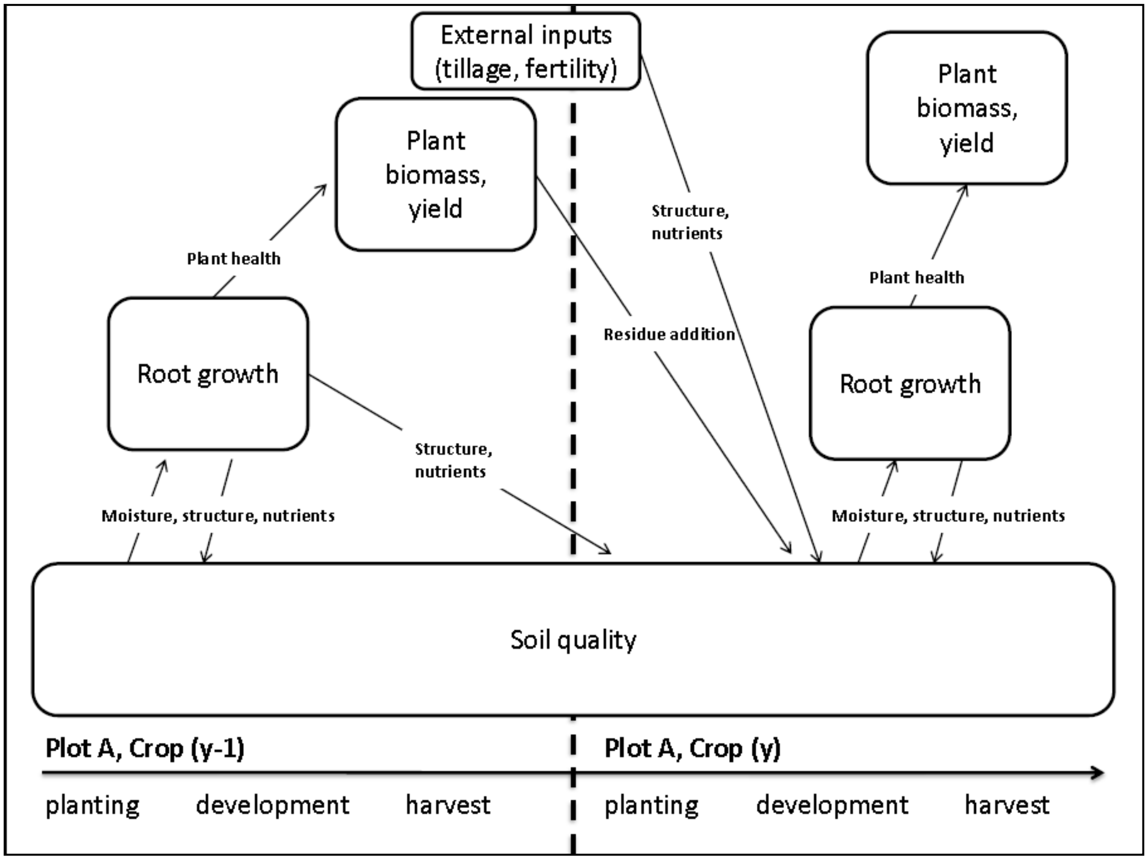

Figure 1. Conceptual framework by which soil and plant parameters from the previous crop (Y-1) are considered both as causal agents contributing to the soil environment for the subsequent crop (Y) and as endpoints assessing the performance of the system under crop Y. Samples taken at planting, in midsummer around maximum root development and at harvest show the environment inherited by crop Y, crop Y's response to this environment, and crop Y's legacy to crop Y+1.

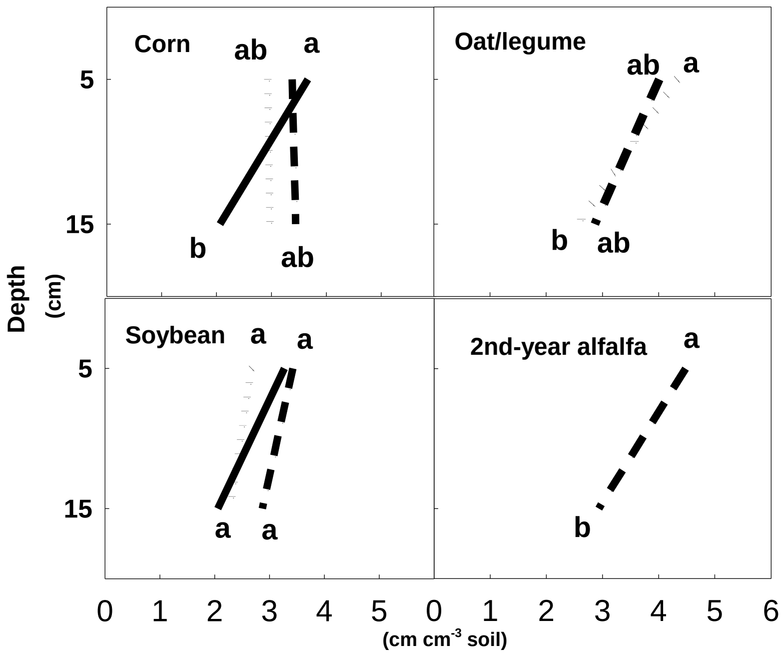

Figure 2. Root length density in summer of 2009, for the 2-yr (—), 3-yr (••••) and 4-yr (– –) rotations. Different letters denote significant differences between rotations and depths within crops at p<0.05.

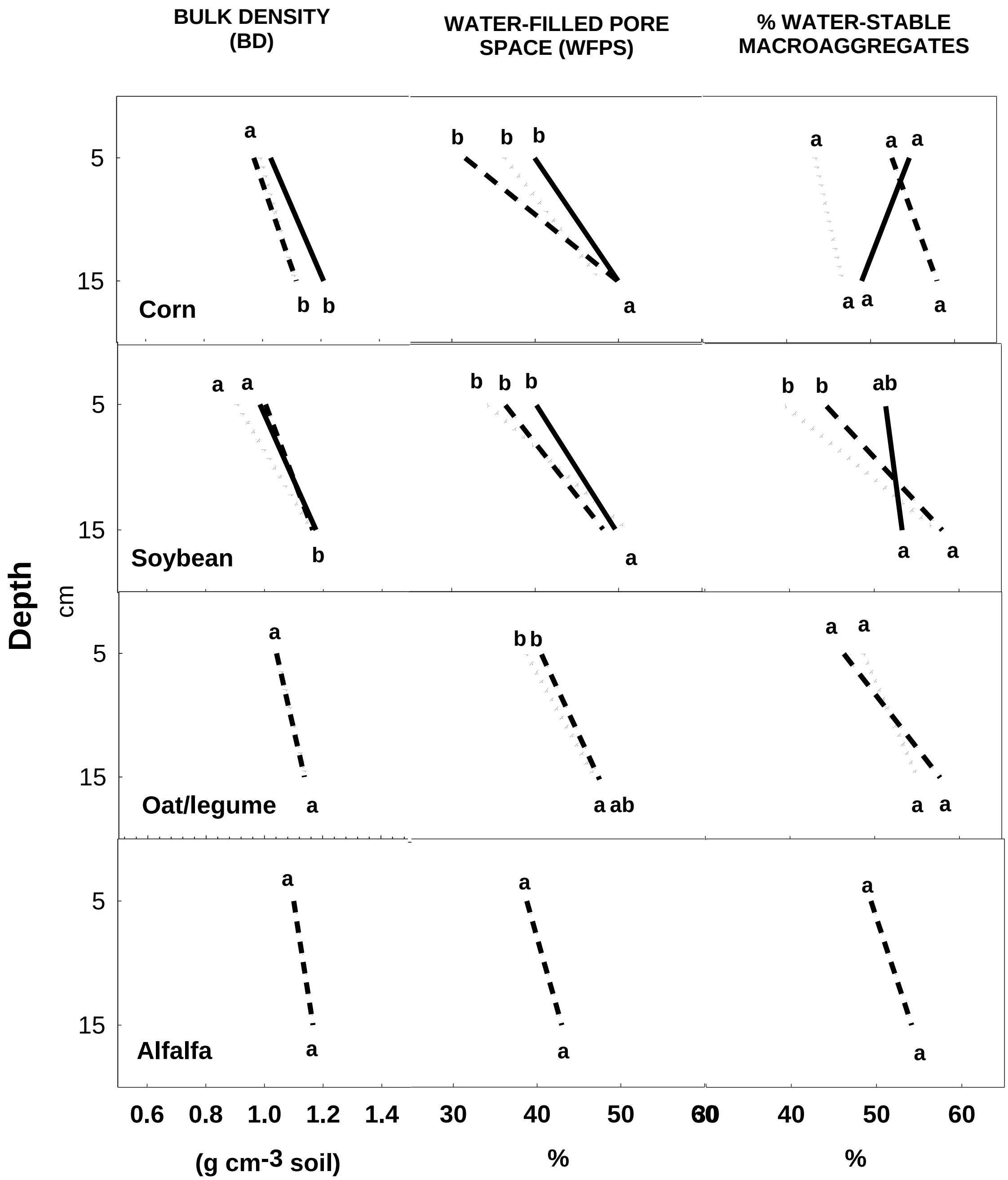

Figure 3. Physical indicators of soil quality by rotation and depth for each crop. Reported values are LSMEANS of spring, summer and fall of 2009 values. Different letters denote significant differences within crops between rotations and depths at $p<0.05$. Clay was included as a covariate where it was significant at $p<0.05$.

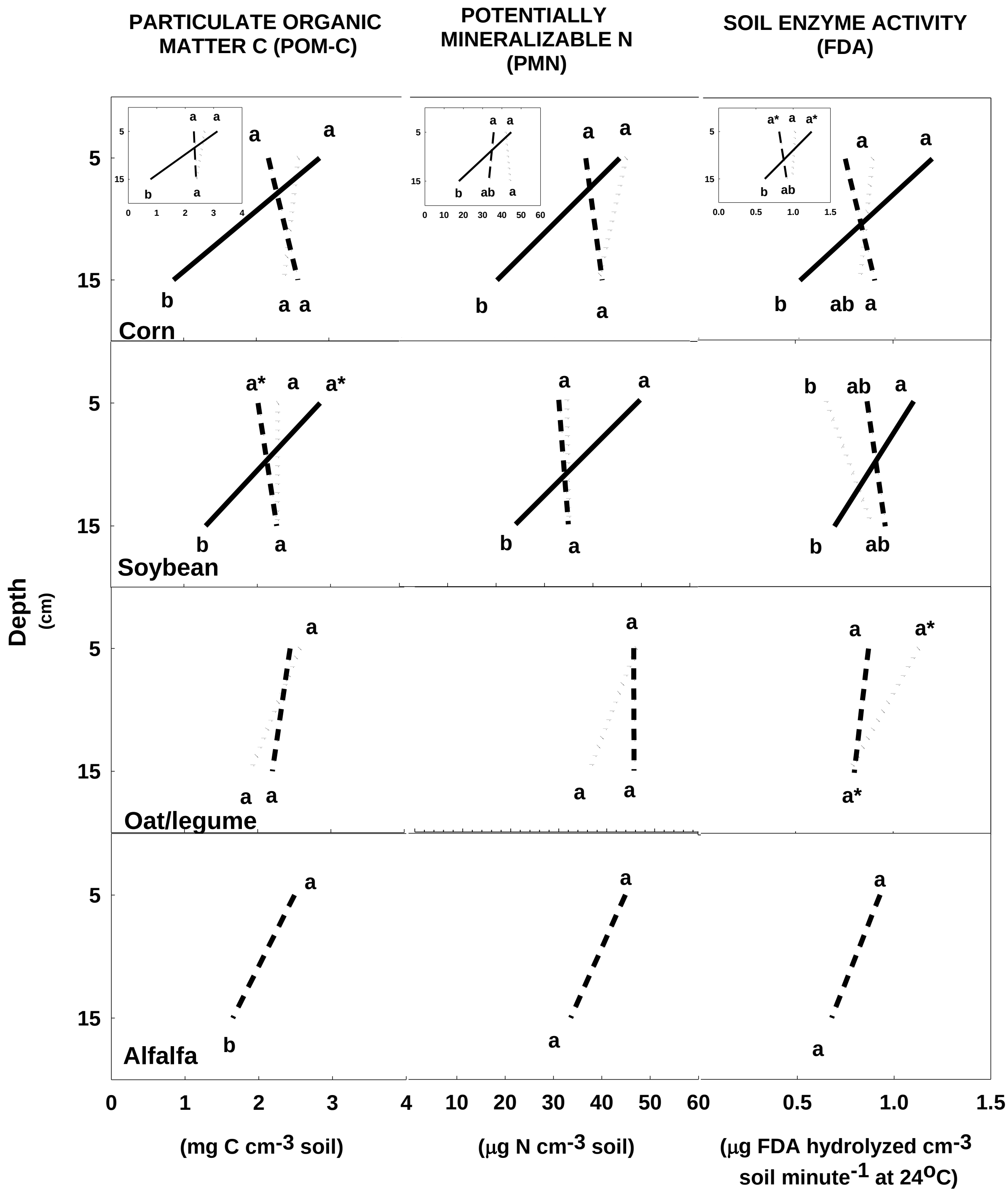

Figure 4. Soil chemical and biological indicators plotted by rotation and depth for each crop. Reported values are LSMEANS of spring, summer and fall of 2009 values. Means followed by different letters denote significant differences within crops between rotations and depths at $p<0.05$. Clay was included as a covariate. Asterisks denote marginally significant ($p<0.10$) differences between the marked values. Insets show LSMEANS of spring 2009 and 2010.